John von Neumann Institute for Computing

Secondary Structure of Compstatin Analogues: Insights from Molecular Dynamics Simulations in Explicit Water

Phanourios Tamamis, Georgios Archontis

published in

NIC Workshop 2006,
From Computational Biophysics to Systems Biology,
Jan Meinke, Olav Zimmermann,
Sandipan Mohanty, Ulrich H.E. Hansmann (Editors)
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **34**, ISBN-10: 3-9810843-0-6,
ISBN-13: 978-3-9810843-0-6, pp. 133-136 , 2006.

http://www.fz-juelich.de/nic-series/volume34

Secondary Structure of Compstatin Analogues: Insights from Molecular Dynamics Simulations in Explicit Water

Phanourios Tamamis[1] **and Georgios Archontis**[1]

Department of Physics, University of Cyprus, PO20537, CY1678, Cyprus
E-mail: archonti@ucy.ac.cy

Compstatin is a cyclic, 13-residue peptide, which binds to the protein C3 and inhibits the activation of the complement system. To obtain a better understanding of the structural properties of the free peptide and the relation between sequence, structure and activity, we conduct multi-ns explicit-water simulations of three related systems. i) A native analogue of compstatin that is acetylated at the N-terminal end (NAT), ii) the inactive single mutant Q5G (G5), and iii) the more active double mutant V4W/H9A (W4A9). In the simulations the 5–8 region adopts a β-turn conformation with high probability, in agreement with earlier NMR studies. The propensity of this turn is not correlated with the activity of the three analogues. The rest of the molecules are mostly in a random-coil state, with β-turns occasionally formed outside the region 5–8.

1 Introduction

The complement system constitutes the initial defense against foreign pathogens[1]. The development of drugs controlling complement activation is of significant medical interest, as it could treat a number of pathological conditions, including rheumatoid arthritis and rejection of xenotransplantation[2]. A candidate against the unregulated activation of the complement system is the cyclic peptide compstatin, which has the sequence Ile1-Cys2-Val3-Val4-Gln5-Asp6-Trp7-Gly8 -His9-His10-Arg11-Cys12-Thr13-NH_2 and contains the disulfide bridge Cys2–Cys12 (see fig. 1, left panel). Earlier NMR studies of the free peptide showed that the segment 5–8 adopts a predominantly type-I β-turn conformation in solution, whereas the rest of the molecule is highly flexible[3]. Additional systematic mutational studies determined that the two cysteines 2 and 12, Val3 and the residues 5–8 of the β-turn are critical for activity, but the turn itself is not sufficient for activity. In contrast, residues Val4, His9, His10 and Arg11 are not required for activity[4,5].

Apart from the formation of the 5–8 β-turn, the information from the NMR spectra on intramolecular interactions is limited, due to extensive conformational averaging. To gain insight on these interactions, we conduct multi-ns molecular dynamics simulations of a native analogue (NAT) (acetylated at the N-terminal end and more active by a factor of three), the single inactive mutant Q5G (G5)[3], and the double mutant V4W/H9A (W4A9), which has a 45-fold higher activity than the native compound[4,5].

2 Methods

The simulation methodology has been described in detail in ref.[6]. Briefly, the total system consisted of one peptide and 7921 water molecules (23975 atoms in the case of NAT). The interatomic interactions were taken from the CHARMM22 all-atom force field[7] and the water was represented by a modified TIP3P model[8]. All simulations employed cubic

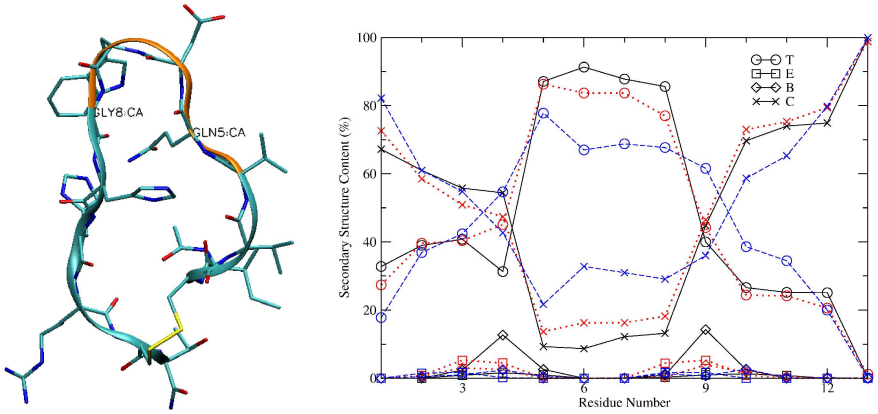

Figure 1. **Left:** Typical simulation structure of NAT. **Right:** Average residue secondary-structure population. The continuous, dotted and long-dashed lines correspond, respectively, to G5, NAT and W4A9.

boundary conditions and were performed with the program CHARMM[9], version c31b2. For NAT, W4A9 and G5 we conducted, respectively, 22, 16 and 11 independent 2-ns simulations, starting from several different NMR structures (entry 1A1P[3]).

3 Results

To determine the average secondary structure of the three systems, we analyzed snapshots extracted at 5-ps intervals with the algorithm STRIDE[10]. The left panel of fig. 1 shows a representative simulation structure of the NAT analogue. The most important observed secondary structural elements correspond to random coil (C), β-turn (T), β-bridge (B) and β-sheet (E). The right panel of fig. 1 shows the corresponding average populations.

In all three analogues the region 5–8 folds into a β-turn with probability 85% (G5) to 70% (W4A9). The highest turn population corresponds to the inactive peptide (G5) and the lowest to the more active double mutant W4A9. Thus, the 5–8 turn propensity is not proportional to the peptide activity and its formation is not sufficient for activity, as shown by G5. A similar observation has been made with the inactive mutant V3A, which also has a high 5–8 turn propensity[4]. The probability of type I/III turns (family α_R-α_R[12]) is 36.6%–33.5% for NAT, in good agreement with the NMR estimate. In G5 and W4A9 the probability is, respectively, higher and smaller than NAT[6].

There is a small to moderate probability for the occurence of turns elsewhere in the sequence. The most important of these is formed by the segment 6–9 in analogue W4A9, and is usually fused with the turn 5–8. Additional β-turns do occur in segments 1–4, 2–5, 8–11 and 9–12, but with significantly lower probability[6]. The N-terminal (1–4) and C-terminal (9–13) regions are largely unstructured. This is in agreement with the observed

conformational averaging in the NMR spectra, which has demonstrated that the region outside 5–8 is highly flexible.

Earlier MD simulations with a generalized Born approximation for the solvent[11] and a polar hydrogen energy function[9] suggested the frequent formation of β-hairpin and α-helical motifs[12]. In the current simulations, the 3–9 region of NAT forms a β-hairpin with a $\approx 5\%$ probability (fig. 1). In the other two analogues, the corresponding probability is 1.7% (W4A9) and 1.5% (G5). The inactive (G5) analogue forms the two main chain hydrogen bonds Val3(O)-His10(N) and Gln5(N)-Gly8(O), creating an isolated bridge between residues 4 and 9 (fig. 1). We conducted test simulations of the NAT system with the same GB energy function[9, 11, 12], in which the peptide formed β-hairpin and α-helical elements lasting for tens of ns (not shown). Such discrepancies between explicit and implicit solvent treatments have been observed with various GB implementations[13, 14], possibly due to overstimation of the intramolecular interactions by GB. At the same time, the current explicit solvent simulations could underestimate somewhat the formation of β-hairpin and α-helical elements (despite their success in reproducing the NMR 5-8 β-turn population), due to the finite length of the simulations and force-field inaccuracies.

In conclusion, our simulations suggest that the free peptides are flexible, with a propensity for a 5–8 β-turn which is not proportional to activity and is highest for the inactive peptide (G5). The remainder of the molecule is predominantly in a random coil state, with additional β-turns occasionally observed outside the region 5–8.

Acknowledgments This work was partly funded by the ERYAN/0603 grant. We acknowledge useful discussions with Profs. Spyros Skourtis, Dimitrios Morikis, and John Lambris.

References

1. H. J. Muller-Eberhard. *Complement-Chemistry and pathways*. Raven Press, New York, 1992.
2. A. Sahu and J. D. Lambris. *Immunopharmacology*, 49:133–148, 2000.
3. D. Morikis, N. Assa-Munt, A. Sahu, and J. D. Lambris. *Prot. Sci.*, 7:619–627, 1998.
4. D. Morikis, M. Roy, A. Sahu, A. Troganis, P. A. Jennings, G. C. Tsokos, and J. D. Lambris. *J. Biol. Chem.*, 277:14942–14953, 2002.
5. B. Mallik, M. Katragadda, L. A. Spruce, C. Carafides, C. G. Tsokos, D. Morikis, and J. D. Lambris. *J. Med. Chem.*, 48:274–286, 2005.
6. P. Tamamis, S. Skourtis, D. Morikis, J. D. Lambris, and G. Archontis. Conformational analysis of compstatin analogues with molecular dynamics simulations in explicit water. In preparation.
7. A. D.Mackerell et al. *J. Phys. Chem. B*, 102:3586–3616, 1998.
8. E. Neria, S. Fischer, and M. Karplus. *J. Chem. Phys.*, 105:1902–1921, 1996.
9. B. R. Brooks et al. *J. Comput. Chem.*, 4:187–217, 1983.
10. D. Frishman and P. Argos. *PROT*, 23:566–579, 1995.
11. B. Dominy and C. .L. Brooks III. *J. Phys. Chem. B*, 103:3765–3773, 1999.
12. B. Mallik, J. D. Lambris, and D. Morikis. *Proteins: Struct., Func. and Bioinf.*, 52:130–141, 2003.
13. R. Zhou and B. Berne. *Proc. Natl. Acad. Sci. USA*, 99:12777–12782, 2002.
14. H. Nymeyer and A. Garcia. *Proc. Natl. Acad. Sci. USA*, 100:13934–13939, 2003.